ARTIFICIAL INTERSPECIFIC HYBRIDIZATIONS IN MELAMPODIUM SECTION ZARABELLIA (COMPOSITAE)

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Abstract

Interspecific hybridization experiments were completed for three of the five species in *Melampodium* section *Zarabellia*. Hybrids between the two diploid species, *M. gracile* ($n = 9$) and *M. microcephalum* ($n = 9$) showed normal bivalent pairing at metaphase I but were highly sterile. The observation of an occasional quadrivalent during diakinesis in these hybrids indicates that at least one reciprocal translocation exists between the genomes of these two diploid species. Artificial hybrids between *M. paniculatum*, a tetraploid species with $n = 18$, and the two diploid species were triploid and sterile. Analysis of pairing relationships in the triploid hybrids indicates that perhaps one, but not both, of the diploid species contributed a genome to tetraploid *M. paniculatum*.

Melampodium (Compositae, Heliantheae) of Latin America contains 37 species that are classified into six taxonomic sections (Stuessy, 1972). Of these, sect. *Melampodium* has received the most intensive study to determine evolutionary relationships. Particular emphasis has been placed on the white-rayed complex of series *Leucantha*, in which detailed cytological and chemical studies have been completed (Stuessy, 1971a; Stuessy et al., 1975; Fishback et al., 1976). This paper summarizes evolutionary studies on sect. *Zarabellia* (Cass.) DC., which is distributed primarily in Mexico and Central America and contains five species (Stuessy, 1972): *M. longifolium* Cerv. ex Cav.; *M. mimulifolium* Robins.; *M. gracile* Less.; *M. microcephalum* Less., and *M. paniculatum* Gardn. The last three species are unique within the section in possessing an outer involucre of three phyllaries, and stipitate-glandular hairs. For this reason the three taxa are believed to be related very closely.

Melampodium microcephalum, M. gracile, and *M. paniculatum* are differentiated morphologically by a number of features (Figs. 1–6; Stuessy, 1972). In general, *M. paniculatum* is the most easily distinguished of the three species. The other two species are separated most easily by the subauriculate leaf bases in *M. gracile* versus the attenuate to obtuse leaf bases in *M. microcephalum*. With regard to distributions, *M. paniculatum* is confined primarily to Central America, whereas the other two species are principally Mexican (Fig. 7). Within Mexico, *M. gracile* and *M. microcephalum* have partially overlapping ranges especially in the states of Michoacán and Morelos. Chromosomal numbers of *M. gracile* and *M. microcephalum* are $n = 9$, and *M. paniculatum* is known usually as $n = 18$ (Stuessy, 1971b, 1971c; Figs. 8–15). A hexaploid ($n = 27$) has been reported in this species

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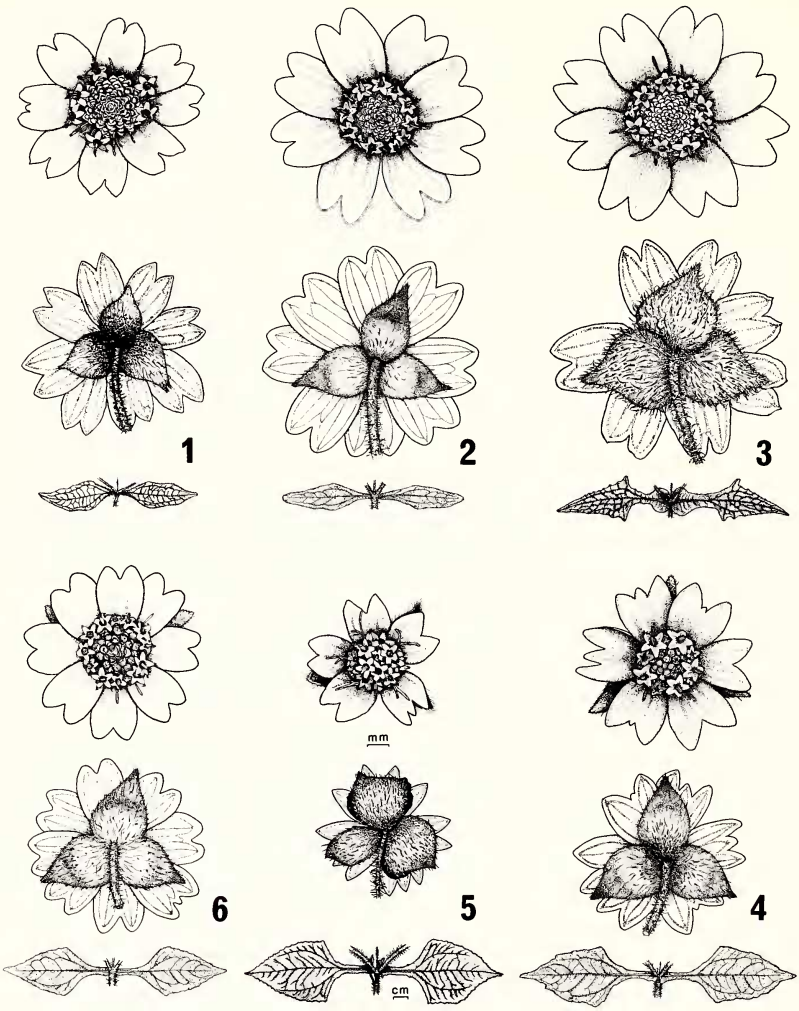


FIGS. 1–6. Representative heads (both top and bottom view) and mature leaves of species and F_1 hybrids of *Melampodium*. All same scale. 1. *M. microcephalum, Stuessy 681.* 2. *M. gracile* × *M. microcephalum.* 3. *M. gracile, Stuessy 537.* 4. *M. gracile* × *M. paniculatum.* 5. *M. paniculatum, Stuessy 579.* 6. *M. paniculatum* × *M. microcephalum.*

from a population in Brazil (Coleman, 1970). These diploid and polyploid chromosomal levels plus morphological similarity suggest a possible polyploid origin for *M. paniculatum* involving one or both of the two diploid species. A program of interspecific hybridizations among all three taxa was initiated to determine patterns of evolution in the section. This paper reports results of these hybridizations.

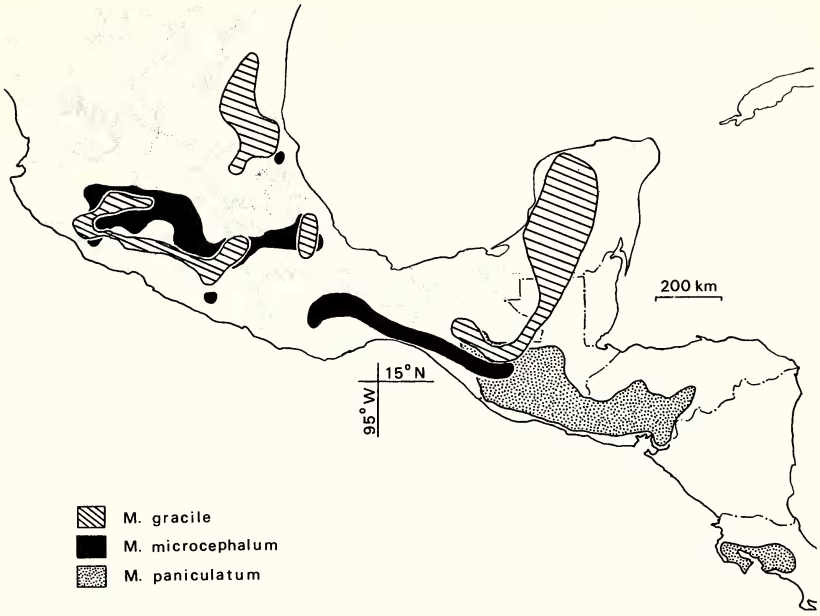
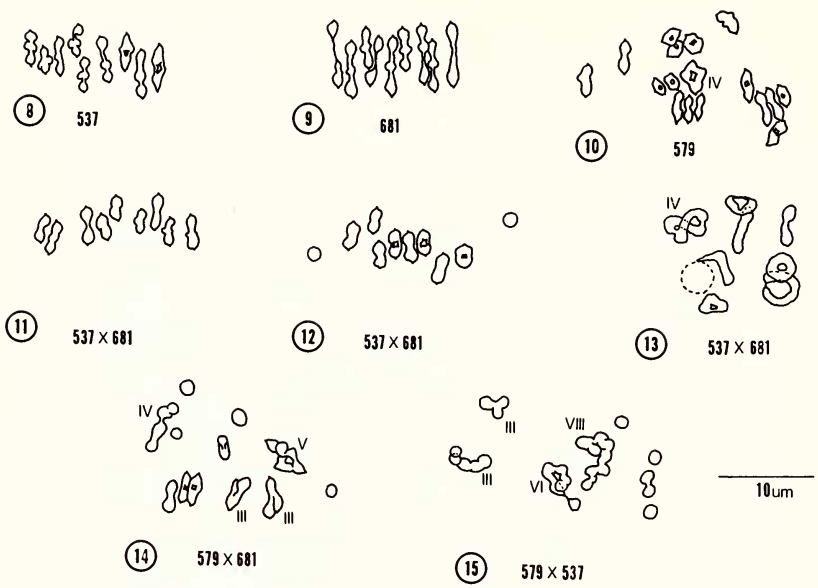


FIG. 7. Generalized distributions in Mexico and Central America of *Melampodium gracile, M. microcephalum,* and *M. paniculatum.* Isolated Colombian and Brazilian localities of *M. paniculatum* not shown.

METHODS

Melampodium is well suited for controlled crossing experiments because the ray florets are carpellate and fertile, and the disc florets produce copious amounts of viable pollen but have abortive ovaries. Removal of disc florets when young, therefore, results at maturity in a functionally female head of ray florets only. Preliminary interplant crosses within species of sect. *Zarabellia* and within *M. divaricatum* (Rich. in Pers.) DC. of sect. *Serratura* (Stuessy, 1968) indicated that the emasculation technique does not hinder or retard normal development of ray florets.

Seeds produced by open-pollination were gathered in the field and grown in the greenhouse to serve as the parental plants. One population of each was used from the following localities: *M. gracile,* México, Yucatán, 13 km N of Mérida, *Stuessy 537*; *M. microcephalum,* México, Michoacán, 5 km SE of Ciudad Hidalgo, *Stuessy 681*; *M. paniculatum,* Guatemala, Huehuetenango, 3 km S of Huehuetenango, *Stuessy 579.* In the crosses, six plants of *M. gracile* and three plants each of *M. microcephalum* and *M. paniculatum* were used. Voucher specimens of these parental stocks and all hybrids described below are in OS.



FIGS. 8–15. Camera lucida drawings of meiotic chromosomes of species and hybrids of *Melampodium* sect. *Zarabellia*. All same scale. 8. *M. gracile* ($n = 9$, *Stuessy 537*). 9. *M. microcephalum* ($n = 9$, *Stuessy 681*). 10. *M. paniculatum* ($n = 18$ [$2n = 16II + 1IV$], *Stuessy 579*). 11–13. *Melampodium gracile* × *M. microcephalum*. 11. Metaphase I. Nine bivalents. 12. Metaphase I. Eight bivalents and two univalents. 13. Diakinesis. Seven bivalents and one quadrivalent. 14. *M. paniculatum* × *M. microcephalum*. Metaphase I. One pentavalent, one quadrivalent, two trivalents, four bivalents, and four univalents. 15. *M. paniculatum* × *M. gracile*. Metaphase I. One octavalent, one hexavalent, two trivalents, one bivalent, and five univalents.

Lens paper bags were used to cover all heads employed in the crossing program. Preliminary checks were carried out for autonomous apomixis in both parental and hybrid generations by removing the disc florets and bagging the heads. To determine the breeding systems of the three species, selfings of individual heads were completed. Crosses between plants of the same species were accomplished to serve as a background against which to evaluate the interspecific crosses. All crosses were accomplished in greenhouses at The University of Texas and The Ohio State University with conditions of high humidity and a temperature of ca. 27°C. For determining pollen viability, acetocarmine and lactophenol-cotton blue stains were used. A positive pollen grain coloration by the two was assumed to indicate pollen viability (Hauser and Morrison, 1964). A minimum of 500 pollen grains was examined per plant. Because changes in the environment can affect pollen viabilities (Jones, 1976), pollen samples were taken only from greenhouse plants grown under uniform conditions. In both

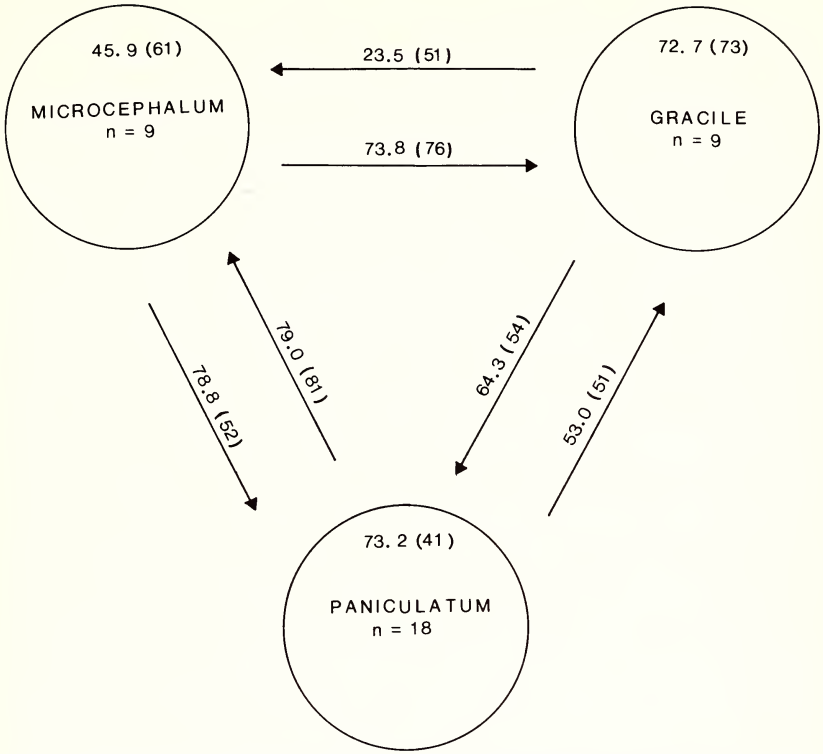


FIG. 16. Summary of crossing relationships within and among *Melampodium gracile, M. microcephalum,* and *M. paniculatum.* Numbers in circles indicate percent seeds obtained in intraspecific crosses. Arrows and numbers indicate percent seeds obtained in interspecific reciprocal crosses (arrows point toward female parent). Numbers in parentheses indicate total number of florets in each type of cross carried out.

cross- and self-pollinations, pollen was dusted on receptive stigmas on three consecutive days to insure effective pollination.

Modified Carnoy's solution (4 chloroform:3 absolute ethanol:1 acetic acid) was used to kill and fix flower buds of both parents and hybrids. Buds prepared in this fashion were refrigerated until processed by the method described by Snow (1963).

RESULTS

Crossing data. Seed set was absent in emasculated heads of *Melampodium gracile* (40 florets/3 plants), *M. microcephalum* (38/3), and *M. paniculatum* (23/3). The absence of any fertile seed indicates that autonomous apomixis is not present in the three populations. The results of self-pollinations indicate that all species are self-fertile to some extent with the tetraploid, *M. paniculatum,* being the most fer-

TABLE 1. SURVIVAL AND FERTILITY IN THE F_1 GENERATION.

Hybrid (♀ × ♂)	Survival Data		Pollen Stainability		Seed Set (# hybrids/ % seed set)	
	# seeds produced	% survival to maturity	# plants examined	% stained (range)	selfed	crossed
microcephalum × *paniculatum*	64	36	36	12 (6–25)	7/0	14/0
paniculatum × *microcephalum*	41	23	13	10 (5–20)	5/0	10/0
paniculatum × *gracile*	36	19	5	6 (2–10)	7/0	7/0
gracile × *paniculatum*	27	10	9	6 (4–10)	4/0	4/0
gracile × *microcephalum*	56	17	13	11 (6–25)	7/0	5/3.6
microcephalum × *gracile*	12	0	—	—	—	—

tile (80.1 percent; 121 out of 151 florets) and the diploids being less so with 15.0 percent (30/200) and 28.8 percent (80/278) in *M. microcephalum* and *M. gracile,* respectively. Relatively high intraspecific crossabilities of approximately 73 percent were found in *M. gracile* and *M. paniculatum. Melampodium microcephalum* had a lowered crossability of 46 percent (Fig. 16).

The interspecific crosses among the three taxa were very successful (Fig. 16). Three principal results were observed. First, the percentages of crossability in three hybrid combinations [*M. gracile* (♀) × *M. microcephalum* (♂); and reciprocals of *M. microcephalum* × *M. paniculatum*] were as high or higher than those of intraspecific crosses. Second, the reciprocal crosses between each pair of species produced equivalent seed set except a lowered seed production between *M. microcephalum* (♀) and *M. gracile* (♂). Third, the highest crossability (79 percent) occurred between the diploid *M. microcephalum* and the tetraploid *M. paniculatum.*

Characteristics of hybrid generation. Seeds produced from all reciprocal interspecific crosses germinated and survived to maturity (Figs. 2, 4, 6) except for *M. microcephalum* (♀) × *M. gracile* (♂) (Table 1). These data closely parallel those of the percentages of hybrid seeds set (Fig. 16). In both instances the highest percentages of hybrid seed produced and the highest percentage of surviving hybrids occur in the reciprocal crosses between *M. microcephalum* (diploid) and *M. paniculatum* (tetraploid). The very small number of fertile seeds pro-

duced in the hybrid progeny indicates again the absence of autonomous apomixis (Table 1).

For the meiotic behavior of the artificially produced hybrids to be analyzed effectively, a few comments need to be made first about the diploid parents. In most cases, meiosis appears normal in the three parental species (Stuessy, 1971b). From diakinesis through metaphase I, nine bivalents are invariably observed in both *M. microcephalum* (Fig. 9) and *M. gracile* (Fig. 8). Some variation in pairing was noted in the tetraploid parent, *M. paniculatum*. Eighteen bivalents were observed in most meiocytes through metaphase I. Occasionally, however, one quadrivalent was formed (Fig. 10). Disjunction and microspore formation in the tetraploid appeared normal. In the hybrid between the two diploid species, *M. gracile* × *M. microcephalum,* meiosis appeared normal with most meiocytes exhibiting nine bivalents from diakinesis through metaphase I (Fig. 11). Two univalents (Fig. 12) were found in 12 of 50 meiocytes observed in metaphase I. These unpaired chromosomes may have been the result of the early disjunction of a bivalent because such univalents were not observed at earlier meiotic stages. At anaphase I, one or two chromosomes occasionally remained on the metaphase plate, and micronuclei were noted in 12 of 236 cells sampled. Both of these irregularities may have been the result of early disjunction. A single quadrivalent (Fig. 13) was seen in two of 50 meiocytes observed at diakinesis, indicating the presence of at least one reciprocal translocation between the two diploid genomes. Such quadrivalents were not detected in approximately 50 cells sampled at metaphase I. Disjunction and tetrad formation appeared normal in most meiocytes of the diploid hybrid.

Chromosomal pairing in the triploid hybrids was highly variable. In the reciprocal hybrids of both *M. paniculatum* × *M. gracile* and *M. paniculatum* × *M. microcephalum,* from a sample of approximately 50 cells of each cross, meiocytes exhibited various combinations of univalents, bivalents, trivalents, and higher order multivalents. Unlike those observed in the diploid hybrid, multivalents in both triploids usually persisted through metaphase I. In *M. paniculatum* × *M. gracile,* one hexavalent and one octavalent were observed in one meiocyte (Fig. 15), indicating the presence of at least three reciprocal translocations among the three diploid genomes. The maximum association observed in *M. paniculatum* × *M. microcephalum* (Fig. 14) was one quadrivalent and one pentavalent, indicating the presence of at least two reciprocal translocations. Disjunction and tetrad formation in both triploid hybrids were highly irregular. Multiple lagging chromosomes were common at anaphase I, and multiple micronuclei were present at the tetrad stage.

The F_1 hybrids of all combinations are highly pollen sterile (2–25 percent stainable grains; Table 1). Pollen stainability in parental plants was 80–95 percent. Among the hybrid progenies, the lowest values

(about 6 percent) prevail in crosses between the diploid *M. gracile* and the tetraploid *M. paniculatum*. The stainability was approximately the same (10–12 percent) in the two other hybrid combinations.

The data from both selfing of individual heads and interplant crosses within the same hybrid type indicate that an F_2 generation is developed only with difficulty. In all of the attempted crosses to produce an F_2 generation, only two seeds were obtained out of 263 florets examined; these were from the diploid hybrid, *M. gracile* × *M. microcephalum*. Frequent observations of flowering heads of all hybrid plants disclosed abortive ovaries. The hybrids as a group, therefore, are male and female sterile. As a result, the development in nature of large numbers of individuals of advanced hybrid generations would be unlikely.

Discussion

Evolutionary relationships

In assessing evolutionary relationships among *Melampodium gracile, M. microcephalum,* and *M. paniculatum* an important caution is that only single populations of each species were used in the crossing program. The interspecific relationships of these species, therefore, have not been determined from crosses among several to many populations from throughout their ranges, as would be desirable. Because of the fragmented nature of the distributions of the diploids (Fig. 7), it is possible that genetic and/or chromosomal divergence has taken place among the isolated populational segregates; such divergence would not have been detected in the present study. Although additional crosses are needed to provide a more complete view, some suggestions can be made concerning evolutionary and taxonomic affinities among these three species based upon available data.

Between the diploids. Although *Melampodium microcephalum* and *M. gracile* have identical chromosome numbers ($n = 9$), they differ in chromosomal rearrangements, including at least one reciprocal translocation. Bivalent formation is normal in most meiocytes of the diploid hybrids, but early disjunction results in two univalents in some cells at metaphase I. However, even when taken together, these results do not account for the observed level of pollen sterility (ca. 89 percent) and low seed set (ca. 4 percent) in interplant crosses of F_1's. Thus additional, cryptic, structural differences or some other genetic imbalance must exist between the two species. If these additional differences are structural (e.g., translocations, inversions) they may not be of sufficient magnitude to influence pairing. However, they could reduce fertility.

The successful yield of interspecific hybrids (Fig. 16) provides evidence that the two diploid species are closely related and of the same evolutionary line within the genus. The geographic distributions of

the diploids plus the available crossing data suggest three alternative hypotheses for their evolution. All three hypotheses assume that the present sympatry in central Mexico occurred after the speciation event(s). (1) One of the species once possessed a wide distribution (*M. gracile* and *M. microcephalum* seem equally plausible), and the other evolved in one region as a peripheral isolate that was then dispersed to other favorable habitats. (2) The same situation prevailed as above, but different derivative isolates originated in different parts of the range of the parent (i.e., there was a polyphyletic origin for one of the diploids). (3) The two taxa evolved from a common ancestor and the present pattern of partial sympatry is the result of range extensions. Of these alternatives, the second possibility seems the least likely because the morphologies of isolated populations within the two diploid species are not very different. The first and third alternatives seem more plausible, but no data are at hand to favor one over the other.

Between the diploids and tetraploid. The level of multivalent formation in the triploid, *M. paniculatum* × *M. microcephalum,* was lower than that found in the triploid, *M. paniculatum* × *M. gracile.* Multivalents in both of these diploids may have two separate origins. First, they may be the product of reciprocal translocations between the single genome from the diploid parent and the two genomes of the tetraploid parent. Second, they may also result from reciprocal translocations between the two genomes of the tetraploid parent. It is true that pairing in the tetraploids is primarily as bivalents. However, such bivalent pairing may be under genetic control (deWet and Harlan, 1972). If genetic control for bivalent pairing is present, it may break down in the triploid hybrids under the influence of the additional genome from the diploid parent. Only in the absence of such genetic control can it be said that the three haploid genomes of the *M. paniculatum* × *M. microcephalum* hybrid are structurally more similar than those in the *M. paniculatum* × *M. gracile* hybrid.

Based upon these data, it cannot be said definitely that either of the diploids contributed to the formation of the tetraploid species. However, they probably were not both involved, and *M. microcephalum* does seem more probable as one of the parents than does *M. gracile.* The former species has fewer observed translocations between it and *M. paniculatum,* and the F_1 hybrids have a higher survival rate, are more robust, and have a higher percentage of viable pollen than in the cross with *M. gracile.*

A program of artificial hybridization among the three species studied here and the other species of sect. *Zarabellia* (*M. longifolium* Cerv. ex Cav. and *M. mimulifolium* Robins.; Stuessy, 1972) would be of value. The former is known cytologically as $n = 9$ (Stuessy, 1971b), but the latter is known only from the type collection (Robinson, 1901) and has not yet been studied chromosomally. Although *M. longifolium* lacks the stipitate-glandular peduncles and three outer phyllaries of

the taxa treated in this paper, the leaves resemble those of *M. paniculatum* and the heads are of similar, small size. *Melampodium longifolium* also is Mexican in distribution and is especially common in San Luis Potosí near where *M. gracile* occurs (Fig. 7).

Taxonomic implications

The two diploid species, *M. gracile* and *M. microcephalum,* are very similar morphologically. They are sufficiently similar, in fact, that difficulty was experienced in sorting some of the herbarium material of the two taxa during the previous revisionary study (Stuessy, 1972). The results of the present investigation reaffirm the closeness of the taxonomic relationship of the two species. At the same time, the documentation of at least one reciprocal translocation between the two taxa, plus sterile F_1 hybrids, supports their recognition as good species. Furthermore, the ease of artificial hybridization of the two diploids and the robust nature of the hybrids suggests that natural hybridization may not be uncommon. This could account for at least some of the difficulty experienced in distinguishing them morphologically.

The cytogenetic data between the diploids and the tetraploid reconfirm the latter's taxonomic distinctness based on morphological, distributional, and cytological grounds. Populations of *M. paniculatum* from Central and South America are morphologically very similar. However, one population of this species from Brazil has been determined to be hexaploid with $n = 27$ (Coleman, 1970). These 6*x* cytotypes probably arose from the tetraploid level through nonreduction during meiosis in one of the gametophytic lines followed by normal fertilization, a common mechanism for the production of higher ploidy levels (Harlan and deWet, 1976). In view of the small sample size examined to date, the presence of tetraploid *M. paniculatum* in South America certainly cannot be ruled out.

Acknowledgments

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